Chapter 4.0

Safety and ecotoxicology of entomopathogenic bacteria

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Abstract: *Bacillus* entomopathogens, especially *Bacillus thuringiensis*, have been used extensively for control of insect pests in crops, forests, and the aquatic environment. Their safety for vertebrates and nontarget invertebrates has been thoroughly documented in a myriad of studies. Their short term effects on nontarget organisms that are unrelated to target insects is negligible. However, the effect of repeated applications on most ecosystems is relatively unknown. It is highly probable that any regular disruption of large insect communities, due to chemical or microbial insecticides or natural disaster, could have long term deleterious effects on higher trophic levels and ecosystem structure. The more diversified the food web, the less likely that complete or partial removal of a single species will result in catastrophic consequences. The more species a given intervention affects, the greater the likelihood of altering ecosystem structure. The safety and environmental impact of entomopathogenic bacteria should be evaluated in light of the risk for nontarget organisms in comparison with other interventions and the effect no treatment at all will have on an ecosystem.

1. INTRODUCTION

Entomopathogenic spore forming bacteria, most notably *Bacillus thuringiensis* (*Bt*), are the most widely used microbial pest control agents (MPCA). The broad spectrum of susceptible hosts, production on artificial media and ease of application using conventional equipment have resulted in widespread use against several insect pests in crops, forest and aquatic habitats.

In order to register a *Bacillus* species as a microbial insecticide, a series of studies must be conducted that assess the toxicity and infectivity of the candidate organism to a designated group of invertebrate and vertebrate nontarget organisms (NTOs). The emphasis of these studies has traditionally been direct effects, typically assessed in one month laboratory studies or one season field studies. Recently, concerns have been raised about long term indirect effects on NTOs when the pest species controlled by the *Bacillus* microbial insecticide becomes unavailable as a source of food. Additionally, questions have been raised about the vulnerability of endangered species of NTO to these insecticides. In the following pages we will highlight studies that address all of these safety issues and attempt to place these data in perspective.

The safety of *Bacillus* entomopathogens for NTOs has been addressed by a number of researchers over the past 50 years. The literature before 1989 on their direct effects on specific NTOs was reviewed in several chapters in *Safety of Microbial Insecticides* [49]. Research on the safety and environmental impact of entomopathogenic *Bacillus* spp. that has been conducted since 1989 will be emphasized in this chapter.

2. DIRECT EFFECTS OF *BACILLUS* ENTOMOPATHOGENS ON NTO INVERTEBRATES

2.1 *Bacillus thuringiensis* (*Bt*)

Here we address varieties of *Bt* that do not produce the β-exotoxin. Because of its toxicity to numerous NTOs including vertebrates, all commercial formulations intended for use in crops, forests and aquatic systems no longer contain β-exotoxin. The reader interested in β-exotoxin should refer to the reviews of Sebesta et al. [76] and Melin and Cozzi [56]. Varieties of *Bt*, the insecticidal activity of which is based on Cry toxins (also known as δ-endotoxins), are now commercially available for use against a wide variety of insect pests including species of Lepidoptera, Coleoptera and

Diptera. The individual Cry toxins are for the most part active against single orders of insect pests and may affect one to several families within an order. There are exceptions such as the Cry2 toxins which are active against certain families of Diptera and Lepidoptera. The specificity of toxins is determined by the molecular configuration of the toxin and the physiology of the host midgut and presence of toxin receptors on the midgut epithelium [20, 75].

The order with the broadest spectrum of families affected by *Bt* toxins, most notably Cry1 toxins, is the Lepidoptera. Discovery and development of hundreds of lepidopteran-active isolates and subsequent genetic manipulation of some has resulted in production of highly efficacious biopesticides for control of several lepidopteran pests of crops and forest. The vast majority of non-lepidopteran NTOs are not directly affected by exposure to commercial products or purified Cry toxins used for control of lepidopteran pests [43, 53, 56, 83, 84]. There are some exceptions that will be presented in subsequent sections of the chapter.

Concerns over the impact of *Bt* products on nontarget Lepidoptera is predominantly focused on indigenous species found mainly in forest habitats or habitats peripheral to agroecosystems. Laboratory bioassays are the starting point to determine possible untoward effects on nontarget Lepidoptera, but they may not always accurately reflect the level of impact in nature. However, phenomena that may be difficult to quantify in the field may be more readily assessed in the lab. For example, Peacock et al. [70] evaluated the effects of two formulations of *Bt* on 42 species of native Lepidoptera and demonstrated differential susceptibility due to species and larval age. They also showed that larvae surviving sublethal dosages of *Bt* were likely to reach adulthood.

Measurement of direct effects in field situations is the most reliable method for determining the impact of a *Bt* spray program on nontarget Lepidoptera. Several researchers have reported on the susceptibility of nontarget Lepidoptera in forest that had been treated with *Bt* [25, 39, 60, 73, 90, 95]. Beneficial and endangered lepidopteran species are among those reported at risk [5, 25, 38]. In addition to effects within treatment zones, Whaley et al. [95] demonstrated that drift of aerially applied *Bt* subsp. *kurstaki* (*Btk*) for gypsy moth control, killed nontarget Lepidoptera as much as 3000 m from the application site.

Effects of *Bt* isolates with activity toward Lepidoptera have negligible effects on insects in aquatic habitats at operational dosages [17]. Kreutzweiser et al. [42] observed no significant effect of high concentrations of *Btk* on drift and mortality of Ephemeroptera, Plecoptera, and Trichoptera. However, they observed 30% mortality in the plecopteran, *Taeniopteryx nivalis*, exposed to the massive concentration of 600 iu/ml for 24 hours.

Mortality of species from pristine lotic habitats may be due to the effects of turbidity and formulation components rather than to *Bt* toxins.

Predators that are exposed to prey that were fed lepdiopteran-active *Bt* have not been shown to be susceptible to *Bt* toxins [7, 100], but *Chrysoperla carnea* that were fed directly on purified Cry1Ab toxin in diet, responded with 57% mortality compared to 30% control mortality [29]. Several additional studies on the effect of *Bt* on predators and other NTOs are summarized by Melin and Cozzi [56] and the USDA Forest Service [89].

There has been less field testing of beetle active isolates containing Cry3 toxins against NTOs. Field trials of *Bt* subsp. *tenebrionis* (*Btt*) in combination with the predatory bug, *Perillus bioculatus*, for control of Colorado potato beetle, *Leptinotarsa decemlineata*, demonstrated compatibility between the bacterium and predator [9, 32]. In another study, five weekly applications of low and high label rates of a genetically engineered isolate of *Bt* for control of *L. decemlineata*, resulted in fair to good control of the beetle with no detectable effects on NTOs including predatory Hemiptera [46]. In contrast, few or no predatory Hemiptera were observed in plots treated with the systemic carbamate insecticide, aldicarb [46]. Giroux et al. [21] reported on the negative effects of beetle-active *Btt* on duration of development of *Coleomegilla maculata* (Coccinellidae) larvae, but concluded it did not cause mortality. Langenbruch [51] reported on the lack of untoward effects of *Btt* on other predators in the potato agroecosystem.

Isolates of *Bt* with Cry4 toxins (*e.g. Bt* subsp. *israelensis* [*Bti*] and others) are highly active against mosquitoes and black flies [48] and have been shown to kill dipteran larvae in closely related families in the sub-order Nematocera, such as certain chironomid, tipulid and blepharocerid species [2, 8, 26, 47, 61, 97]. Numerous bioassays and field trials of *Bti* against NTOs other than Nematocera have demonstrated that the vast majority are not directly affected by *Bti* toxins [8, 27, 36, 45, 47, 58, 97]. Filter feeding species are the most likely to capture and concentrate the parasporal crystals of *Bti* and formulation components that may be harmful. Wipfli and Merritt [97] produced mortality in the filter-feeding mayfly, *Arthroplea bipunctata*, at 500 times the concentration required for black fly control. Increased drift of black flies and NTOs, such as species of Ephemeroptera, Plecoptera, Trichoptera, and Blepharoceridae following treatment with *Bti* has been reported by some authors ostensibly due in part to increased turbidity and formulation components [2, 12, 47, 97].

Under most conditions the majority of predators of mosquitoes and black flies are not susceptible to *Bti*. However, predaceous mosquito larvae in the genus *Toxorhynchites* are susceptible to *Bti* when fed on prey larvae that have been exposed to the bacterium [44, 52]. Because of the lack of direct

deleterious effects in other predatory insects, *Bti* is an ideal microbial insecticide for use in integrated control programs. Although its larvicidal activity is short lived in most habitats [48], undisturbed predators can continue suppression of target insects [1, 47, 66].

2.2 Direct effects of other *Bacillus* entomopathogens

Relatively few studies have been conducted on the effects of *Bacillus sphaericus* and *B. popilliae* on nontarget invertebrates. Both organisms are considerably more specific in their host spectra than *Bt*.

Bacillus sphaericus is an entomopathogen of mosquitoes, but has a markedly narrower mosquito host range than *Bti* and does not appear to directly affect nontarget fauna including chironomids and other Nematocera [1, 47, 63, 91,101]. The bacterium is attractive because of elevated activity against *Culex* and *Psorophora* species and its greater persistence in organically enriched larval habitats [62, 67]. Recycling of *B. sphaericus* in larval cadavers has been reported or suspected, further extending its activity and persistence in the environment [11,40, 67]. Spores may be returned from inaccessible substrates by feeding activity of NTOs which are not harmed by the bacterium [40, 101].

The efficacy of *B. sphaericus* against certain mosquito species, persistence in larval habitats and compatibility with predators has provided extended control in certain circumstances [1, 47].

Isolates of *B. popilliae* are specific pathogens of the Scarabaeidae with no demonstrated effects on NTOs [16, 69]. Their lethal activity is based on septicemia in the host and not on the production of toxin [6, 16] as is the case with *B. thuringiensis* and *B. sphaericus*. Although spores of *B. popilliae* may persist for protracted periods of time in the soil, they only germinate, grow and sporulate in nature within scarab hosts.

3. INDIRECT EFFECTS OF *BT* ON NONTARGET INVERTEBRATES

The indirect effect of *Bt* on nontargets can be broadly divided into two categories: immediate impact and longer term impact. The implications of longer term impact will be addressed under section 6 of this chapter. The immediate indirect effects are most often observed in insects that prey upon or parasitize targeted insects.

Parasitoids are most commonly affected by premature death of the host before development can be completed [3, 4]. Brooks [4] reviewed the literature on host-parasitoid-pathogen interactions and presented several

examples of parasitoids that were unable to complete development due to death of their hosts caused by *Bt* and *B. popilliae*. The effects of *Bt* treatment of host insects on survival and percentage parasitism depends on the host, timing of applications and dosage of *Bt*. Nealis and van Frankenhuyzen [65] noted that spruce budworm larvae, *Choristoneura fumiferana*, that are parasitized by *Apanteles fumiferanae* (Braconidae) are more likely to survive exposure to *Bt* because they feed less. However they did observe a 50-60% reduction in parasitoid populations when the host was treated with *Bt* as third instars. They observed better parasitoid survival when late fourth instars were treated and concluded that *Bt* would complement the beneficial effects of *A. fumiferanae*. A benefit to ingestion of sublethal dosages by host insects is an extended period of development and increased exposure to parasitoids [94].

The effects of host removal on the survival of predators will depend on the specificity of the predator, and the availability of other prey. Studies conducted in aquatic habitats demonstrate some changes in feeding habits of two species of Plecoptera. *Acroneuria lycorias*, a generalist predator, preferred live larvae, but after treatment of prey populations (simuliids) with *Bti* will feed on dead larvae and may exploit other food sources [59]. The total prey ingested by *A. lycorias*, however, declined after treatment of streams with *Bti* [96]. The detrivore, *Prostoia completa*, prefers dead larvae and was not affected by *Bti* treatments [59].

Synergistic and antagonistic activity between *Bt* and other entomopathogens has been reported. Koppenhöfer and Kaya [41] demonstrated synergistic activity between *Bt* subsp. *japonensis* and the entomopathogenic nematodes, *Heterorhabditis bacteriophora* and *Steinernema glaseri* for control of white grubs. A decrease in the incidence of nucleopolyhedrovirus infections has been reported in forests treated with *Btk* [93, 99].

4. EFFECTS OF *BACILLUS* ENTOMOPATHOGENS ON VERTEBRATES

Vertebrate safety testing traditionally refers to a series of tests designed to evaluate the infectivity and pathogenicity of a candidate MPCA. Initially, tests evaluating infectivity and pathogenicity were additions to the standard protocols used to evaluate the toxicity of chemicals, but considerable evolution of these tests has occurred over the past 40 years. An example of this process is the elimination of long term (two year) feeding studies from the evaluation of MPCAs, because these tests were designed to assess

carcinogenicity and this is not applicable. Infectivity is a concern unique to the evaluation of the safety of entomopathogenic bacilli.

Current MPCA tests are typically short term (< one month), and evaluate infectivity using a high dose of the MPCA and include an invasive route of exposure such as intravenous or intraperitoneal injection [79, 80]. If mortality occurs, it must be judged in the context of the test administered. For example, mortality following intracerebral injection of rats with 2×10^8 colony forming units (cfu) of *Btk* is not surprising but would be cause for concern if it occurred after ingestion [79, 80]. A finding of acceptable risk does not mean that under every circumstance a product will never prove harmful. Burges [6] stated that "Registration of a chemical is essentially a statement of usage in which the risks are acceptable. The same must apply to biological agents". Even when products have successfully cleared these hurdles, new questions can arise based on the changing public perception of risk. Questions have been raised periodically concerning the susceptibility of immunosuppressed individuals to *Bt* products [18].

What is the proper way to design safety tests to address this issue, or is it even necessary? There has been considerable debate about the value of safety tests employing immunosuppressed animals. Those opposing this type of safety testing contended that immunosuppressed individuals would succumb to a variety of opportunistic agents before they would become infected by an entomopathogen and furthermore, interpreting data from immunosuppressed animal studies is problematic [6]. In contrast, Shadduck [77] advocated a philosophy of testing known as maximum challenge, which included the use of immunosuppressed or immune deficient animals. Shadduck noted however, that a disadvantage of this approach is that a potentially useful organism may be unfairly labelled as unsafe based on a single test and emphasized that hazard evaluations must be based on a series of tests. A recent study illustrates the difficulty in interpreting test results using immunosuppressed animals [28]. This study reported that *Bt* subsp *konkukian* was infectious when injected subcutaneously (10^7 cfu) in cyclophosphamide-injected mice. However, the mice were only followed for two days after injection and the alleged infection did not become systemic. This study underscores the caveat that a single test cannot be used to determine the hazard of an entomopathogen.

4.1 Direct effects of *Bt* on mammals

There have been thousands of research papers published on *Bt*, but there are relatively few published studies on vertebrate safety. That does not mean that research has not been conducted, but rather that these data are proprietary. Initially, one of the main issues raised about the safety of *Bt* was its close relationship to *B. anthracis*. Some feared that it would somehow mutate and become a human pathogen although Steinhaus [88] eloquently rebutted these concerns. More recent questions have centered on the relationship between *Bt* and *B. cereus*. *B. cereus* has been recognized as the causal agent of an increasing number of cases of food poisoning and as a source of ocular infections [14, 37]. Other researchers have reported that various isolates belonging to several *Bt* serotypes produced *B. cereus* enterotoxins [10]. *Bt* production of enterotoxins has been rebutted by studies that have raised questions about the specificity of the *in vitro* test used to detect enterotoxins [78]. Additionally, no evidence of mammalian toxicity has been found in the numerous laboratory safety studies conducted on *Bt* insecticides; many of these tests were designed to assess the presence of toxins with mammalian activity [15]. No evidence of human infection has been found in epidemiologic studies following mass *Bt* forest spraying campaigns [18, 22, 68]. Finally, at this point *Bt* products have been used for decades and numerous people have been exposed; there has been ample opportunity for any negative effects to be recognized.

As early as 1958, Thuricide, a *Bt* subsp. *thuringiensis* insecticide, was granted an exemption from tolerance by the United States Food and Drug administration based on a series of human and animal studies [19]. These studies included serial passage through mice, intraperitoneal injection in guinea pigs, inhalation studies in mice and human volunteers, and short term feeding studies using human volunteers. Various isolates and subspecies of *Bt* were also tested in long term feeding studies, using a daily dose of 10^9 spores per rat per day for 730 days [33] and a daily dose of 10^{12} spores per sheep per day for 150 days [23]. In highly invasive tests, *Btk* and *Bti* were injected into rats intracerebrally with inocula containing as many as 10^6 cfu; no mortality resulted [79]. In contrast, subcutaneous injection of as little as four spores of *B. anthracis* can kill mice [50].

Determining the infectivity of entomopathogenic bacilli is complicated by their biology. Inocula typically used in safety tests contain a mixture of pores and vegetative cells; commercial products may contain both spores and vegetative cells as well. The spores can remain viable in tissue for periods longer than six weeks [79, 80]. This ability to remain viable without multiplying is referred to as persistence [81]. Persistence may cause confusion, if researchers or clinicians regard simple recovery of *Bt* following exposure as synonymous with infection. In assessing safety, it is more useful to regard infection as established when recovery of a MPCA is linked to tissue damage.

There are three well-documented reports associating human infection with *Bt*. In the first case, a farmer was accidentally splashed in the face with a commercial preparation of *Btk*. An ocular ulcer subsequently developed and *Bt* was recovered [74]. In the second case, a laboratory worker accidentally stuck himself with a needle used to resuspend a cell spore crystal pellet of *Bti* and *Acinetobacter calcoaceticus* var *anitratus* [92]. In the final case, a French soldier stepped on a land mine and suffered a traumatic injury to his leg. Twenty-four hours after the blast *Bt* subsp. *konkukian* was recovered from multiple abscesses [28]. When the first two reports are examined critically, one cannot definitively state that *Bt* caused infection. In the first case, viable spores may have persisted in the conjunctival *cul de sac* and been recovered when the eye was swabbed. In the second case, the laboratory worker was exposed to both *Bt* and another bacterium, as well as metabolites in the culture medium. *Bt* and *A. calcoaceticus* var *anitratus* were cultured from the wound, so it is impossible to state that *Bt* alone caused the infection. The only case where *Bt* was clearly the cause of infection was the French soldier. A land mine blast is certainly a worst case scenario and the *Bt* serotype recovered is not used

commercially. Repeated human exposure by this route is unlikely. When one takes into account the tens of thousands of humans exposed to *Bt* products over the past 40 years, we submit that this single clear-cut case of human infection underscores the mammalian safety of *Bt*.

4.2 Direct effects of *Bt* on other vertebrates

In the United States, as part of the testing necessary for registration, *Bt* products were administered orally to mallard ducks (*Anas platyrhynchos*) and northern bobwhite quail (*Colinus virginianus*). The exposure period was five days and the total dose was as high as 1 x 10^{12} cfu/kg; there were no adverse effects reported. Three species of fish were also tested during the registration process, Sheepshead minnow (*Cyprionodon variegatus*), Steelhead trout (*Oncorhynchus mykiss*) and Bluegill sunfish (*Lepomis macrochiurus*). These species were exposed to *Bt* in concentrations as high as 2.87 x 10^{10} cfu/L in a 30-day static renewal test; test solutions were renewed twice weekly. There was no evidence of pathogenicity or infectivity (bacterial recovery 100 times the administered dose). In one study, there was significant mortality among Steelhead trout exposed to *Bt*. The mortality was attributed to the extreme turbidity of the water in the test group. The fish could not see their food, and in turn attacked each other (WHO, personal communication). Data published in refereed journals on direct effects of *Bt* insecticides support the conclusions of these industry studies. Starlings (*Sturnus vulgaris*), white crowned sparrows (*Zonotrichia leucephrys*) and slate-colored junco (*Junco hyemalis*)fed 7.5 x 10^{8} spores of *Bt* experienced no mortality [85]. Caged rock bass (*Ambloplites rupestris*) exposed to *Bt* over a three-day period experienced no mortality [58]. In contrast Snarski [87] reported that larval and juvenile fathead minnows (*Pimephales promelas*) exposed to 2.0 x 10^{6} cfu/ml of *Bti* died within 24 hours. However, the mortality was due to dissolved oxygen depletion by formulation components. Wipfli *et al*. [98] were also able to kill the embryos of Brook trout (*Salvelinus fontinalis*), Brown trout (*Salmo trutta*) and Steelhead trout with *Bti*. Although mortality occurred, it was only achieved using levels 12,000 times the recommended dose rate. Mortality was attributed to the formulation components. In conclusion, there is no evidence from industry and academic studies that *Bt* insecticides are infectious or pathogenic to birds and fish. Formulated *Bt* products can kill fish indirectly by depleting oxygen levels or making it difficult to find food, but to do so must be applied at a level that is many thousand times the recommended label rate.

4.3 Direct effects of *B. sphaericus* on mammals and other vertebrates

B. sphaericus was subjected to the same infectivity and pathogenicity studies as *Bt* for registration in the United States. These data are unpublished, but the tests included inhalation, oral and intraperitoneal exposure to at least 10^6 cfu per test animal. Nontarget vertebrate studies included fish and birds. Additional studies were conducted by researchers funded by the WHO and included intraocular, intracerebral, subcutaneous, oral, intraperitoneal and aerosol exposure [80, 81]. There was no evidence of infectivity or pathogenicity in these studies. Many of these tests emphasized intracerebral injection (as many as 10^7 cfu) because there were reports in the literature associating *B. sphaericus* with fatal human central nervous system infections. It is noteworthy that in all cases, when these human isolates were injected in experimental animals the isolates were uninfectious. The most well documented human isolate of *B. sphaericus* was in fact misidentified; nevertheless, these cases are periodically cited as cause for concern [82]. In conclusion, entomopathogenic isolates of *B. sphaericus* were noninfectious and nonpathogenic in laboratory animal studies that included worse case exposure scenarios such as intraocular and intracerebral injection.

4.4 Direct effects of *B. popilliae* on mammals and other vertebrates

The mammalian safety studies on *B. popilliae* are summarized by Obenchain and Ellis [69]. Test animals included mice, rats, guinea pigs, rabbits, monkeys, starlings, and chickens. Doses as high as 10^8 spores were used in these studies, and there was no evidence of infectivity or pathogenicity. Heimpel [24] reported that a Maryland researcher ate a spoonful of spore dust to demonstrate its safety. There are no published studies on the infectivity and pathogenicity of *B. popilliae* to fish presumably because of its specificity as well the fact that *B. popilliae* runoff into aquatic systems is minimal.

5. INDIRECT EFFECTS OF *BACILLUS* ENTOMOPATHOGENS ON VERTEBRATES

5.1 Indirect effects of *Bt* on mammals and other vertebrates

It is far more difficult to calculate the indirect effects of *Bacillus* based insecticides than direct effects because these determinations must be made in the field. Natural population fluctuations may be confounded with the effect of the microbial insecticide, and the proper time scale for observation may encompass many years. Additionally, one can argue about what is the proper control to include in these studies. Should the control plots be untreated, or is more appropriate to use as the control plots treated with currently used chemical insecticides? In the case of a forest defoliator such as the gypsy moth (*Lymantria dispar*), should the control plots be defoliated? These issues must be addressed when interpreting data on indirect effects.

Numerous published studies of the indirect effects of *Bt* on small mammals and birds concluded that any effects were minor. *Btk*, fenitrothion and aminocarb were applied aerially for control of jack pine budworm and there were no significant differences in abundance of small mammal populations that could be attributed to *Bt* [35]. Spraying *Btk* in forests significantly reduced the proportion of caterpillars brought to the nests by Chestnut-backed chickadees (*Parus rufescens*) but reproductive success and nestling growth was not affected (WHO, personal communication). Nagy and Smith [64] studied the effect of *Btk* aerial application on hooded warblers (*Wilsonia citrina*) and reported that overall, reduction in Lepidoptera larvae due to spraying had minimal effect, although differences in feeding rates occurred for small clutches. Rodenhouse and Holmes [72] studied food reductions in black-throated blue warblers (*Dendroica caerulescens*) using *Bt*, and found that when caterpillar abundance was reduced, the warblers made significantly fewer nesting attempts and that diets of hatchlings included fewer caterpillars. However, clutch size, hatching success, and the number of fledglings/nest did not differ between treated and control sites. Finally, Holmes [30] studied the reproduction and behavior of Tennessee warblers (*Vermivora peregrina*) in forests treated with *Btk* and tebufenozide. Nestling growth and survival were unaffected by either insecticide, although females in the plots treated with tebufenozide spent more time foraging.

5.2 Indirect effects of *B. sphaericus* and *B. popilliae* on mammals and other vertebrates

There are no studies available on the indirect effects of *B. sphaericus*. This is due in part to its recent commercialization, and since it is used to control mosquitoes there is less concern about its impact on forests. If studies are conducted, they will presumably focus on assessing any deleterious effects on fish caused by prey reduction. There are no published studies of the indirect effects of *B. popilliae* as well. In the case of *B. popilliae*, it was registered before current tests were mandated, and it was then grandfathered into existing legislation when the rules changed. As stated above, since *B. popilliae* is not used to treat forests or in aquatic habitats, there have been few concerns expressed about effects on vertebrates.

6. LONG TERM IMPACT OF *BACILLUS* PATHOGENS USED AS MPCAS

There is a paucity of studies that have assessed the long term impact of *Bacillus* entomopathogens on ecosystem community structure. Of the three pathogens addressed in this chapter, *B. thuringiensis* is the best studied in this regard. Naturally occurring *Bt* is found throughout the world in a number of habitats, yet relatively little is known regarding its role in ecosystems. There are three main theories on the role of *Bt* in nature [55]. Varieties of *Bt* have been referred to as entomopathogens, soil organisms, or saprophytic inhabitants of the phylloplane. Although widespread in soil habitats [54], they usually exist in low numbers in soils relative to other soil bacilli such as *Bacillus cereus* and do not germinate and grow in the soil habitat as readily as *B. cereus*. Diverse varieties have also been isolated from leaf surfaces [86] and grain dust [13]. Natural infections in insects are common in certain protected habitats such as grain silos, but epizootics caused by *Bt* are rare. Although spores can persist in soil and aquatic habitats [57, 71], parasporal inclusions are rapidly denatured in the field. However, in protected field settings, insecticidal activity may persist for up to 30 days [39]. Applications of *Bt* bioinsecticides to agroecosystems and other habitats usually do not result in a build up of spores in the environment. A steady decline in the viability of spores is observed, especially those exposed to sunlight [34]. As a microbial control agent, *Bt* is always inundatively applied to infestations of insects and results in rapid kill of target insects usually without recycling. As discussed above, their short term effects on NTOs that are unrelated to target insects is minimal. Although *Bt* has been used

extensively in a variety of habitats, its long term effects on most ecosystems is relatively unknown. Assessment of the long term environmental impact of *Bt* in agroecosystems may be difficult due the short term nature of such systems and the effect of other agricultural practices on community structure. Stable environments such as forests and permanent aquatic habitats provide the best systems in which to monitor the impact of repeated *Bt* treatments.

Large scale control programs that utilize *Bt* for suppression or eradication of pests such as gypsy moth provide an opportunity to monitor long term effects of the bacterium. USDA Forest Service [89] contends that permanent changes in nontarget populations do not appear likely in gypsy moth suppression projects. Suppression treatments normally consist of a single application of *Btk* in the spring when target foliage averages 45% expansion. However, eradication treatments may include 2-3 applications on a yearly basis. Miller [60] observed reductions in species richness in the guild of leaf-feeding Lepidoptera in forest that was treated over a three year period. Other authors also report a decline in species richness and diversity in Lepidoptera in forests after *Btk* treatments [73, 90]. Risks could be highest for univoltine species especially when populations of sensitive species are clumped in a restricted habitat within the treatment zone [60]. Factors that will contribute to the long term impact on individual susceptible species include voltinism, phenology and distribution of the insect, location within the habitat, and the number and frequency of *Bt* applications. Sample et al. [73] concluded that the long term impact of gypsy moth reduction could benefit some native species.

Routine treatment of mosquito breeding sites with *Bti* in several programs around the world is on the increase. Mosquito control efforts in the Rhine Valley of Germany rely exclusively on applications of *Bti* and to a lesser extend, *B. sphaericus*. Becker [Chapter 6.2] reports no long term deleterious effect on NTOs that are monitored as part of the Rhine Valley program. However, long term monitoring of a wetland ecosystem in the United States indicated that initial regular application of *Bti* for control of mosquito larvae did not result in short term changes [26, 27], but after the wetlands were treated with the bacterium for 2-3 years, species diversity and richness declined significantly [26].

Lotic habitats have also been periodically or regularly treated with *Bti* for control of black fly larvae in Africa, Brazil and North America. The longest ongoing use of the bacterium in rivers has been in the Onchocerciasis Control Program in West Africa where it is used as an intervention during the dry season [31]. Although alternation with conventional chemical larvicides during the wet season precludes long term assessment of the individual impact of *Bti*, Dejoux and Elouard [12] contend that there is no

evidence of long term deleterious effects on ecosystems receiving weekly applications during the dry season. However, the structure of the invertebrate community in the Maraoué River in Ivory Coast after one year of treatment with *Bti* was different in some respects from community structure before treatment and after treatment with temephos and chlorphoxim [12]. In the United States, Molloy [61] observed very little effect on NTOs after multiple applications of *Bti* in small streams. The most sensitive nontarget species, a filter feeding chironomid, responded with an average of 23% mortality to a concentration of *Bti* that killed 98% of simuliid larvae. Wipfli and Merritt [96] observed that reduction of simuliid larvae with *Bti* indirectly and differentially affected predators. Specialist predators in black fly-poor environments were most affected, whereas generalist predators were least affected.

7. CONCLUSION

It is highly probable that any regular disruption of large insect communities, due to chemical or microbial insecticides or natural disaster, could have long term deleterious effects on higher trophic levels and ecosystem structure. The more diversified the food web, the less likely that complete or partial removal of a single species will result in catastrophic consequences. The more species a given intervention affects, the greater the likelihood of altering ecosystem structure. The safety and environmental impact of entomopathogenic bacteria should be evaluated in light of the risk for NTOs in comparison with other interventions and the effect no treatment at all will have on an ecosystem. Major defoliation of a forest by a pest insect such as the gypsy moth, for example, may have broader and more intense long term negative effects on the ecosystem than periodic removal of gypsy moths and affected nontarget Lepidoptera. If the complete removal of an introduced pest has deleterious effects on predators that have grown dependent on the pest, but an ecosystem reverts to its original state, should that be considered catastrophic?

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REFERENCES

[1] Aly C & Mulla MS (1987) Effect of two microbial insecticides on aquatic predators of mosquitoes. J. Appl. Entomol. 103, 113-118.

[2] Back C, Boisvert J, Lacoursiere JO & Charpentier G (1985) High-dosage treatment of a Quebec stream with *Bacillus thuringiensis* serovar. *israelensis*: efficacy against black fly larvae (Diptera: Simuliidae) and impact on non-target insects. Can. Entomol. 117, 1523-1534.

[3] Blumberg D, Navon A, Keren S, Goldenberg S & Ferkovich SM (1997) Interactions among *Helicoverpa armigera* (Lepidoptera: Noctuidae), its larval endoparasitoid *Microplitis croceipes* (Hymenoptera: Braconidae), and *Bacillus thuringiensis*. J. Econ. Entomol. 90, 1181-1186.

[4] Brooks WM (1993) Host - parasitoid - pathogen interactions. pp. 231-272 *In:* Beckage NE, Thompson SN & Federici BA, Eds, *Parasites and Pathogens of Insects, Vol 2: Pathogens.* Academic Press, San Diego.

[5] Brower LP (1986) Comment: the potential impact of DIPEL spraying on the monarch butterfly over-wintering phenomenon. Atala 14, 17-19.

[6] Burges HD (1981) Safety, safety testing and quality control of microbial pesticides. pp 738-767 *In*: Microbial control of pests and plant diseases 1970-1980. (Burges HD, ed.). Academic Press, London.

[7] Cameron EA & Reeves RM (1990) Carabidae (Coloeptera) associated with gypsy moth, (*Lymantria dispar* (L.) Lepidoptera: Lymantriidae), populations subjected to *Bacillus thuringiensis* Berliner treatments in Pennsylvania. Can. Entomol. 122, 123-129.

[8] Charbonneau CS, Drobney RD & Rabeni CF (1994) Effects of *Bacillus thuringiensis* var. *israelensis* on nontarget benthic organisms in a lentic habitat and factors affecting the efficacy of the larvicide. Env. Toxicol. Chem. 13, 267-279.

[9] Cloutier C & Jean C (1998) Synergism between natural enemies and biopesticides: a test case using the stinkbug *Perillus bioculatus* (Hemiptera: Pentatomidae) and *Bacillus thuringiensis tenebrionis* against Colorado potato beetle (Coleoptera: Chrysomelidae). J. Econ. Entomol. 91, 1096-1108.

[10] Damgaard PH, Larsen HD, Hansen BM, Bresciani J & Jorgensen K (1996) Enterotoxin-producing strains of *Bacillus thuringiensis* isolated from food. Lett. Appl. Microbiol. 23, 146-150.

[11] Davidson EW, Urbina M, Payne J, et al. (1984) Fate of *Bacillus sphaericus* 1593 and 2362 spores used as larvicides in the aquatic environment. Appl. Environ. Microbiol. 47, 125-129.

[12] Dejoux C & Elouard J-M (1990) Potential impact of microbial insecticides on the freshwater environment, with special reference to the WHO/UNDP/World Bank, Onchocerciasis Control Programme. pp. 66-83 *In*: Laird M, Lacey LA & Davidson EW, Eds. *Safety of Microbial Insecticides*. CRC Press, Boca Raton.

[13] DeLucca AJ, Palmgren MS & Ciegler A (1982) *Bacillus thuringiensis* in grain elevator dusts. Can. J. Microbiol. 28, 452-456.

[14] Drobniewski FA (1993) *Bacillus cereus* and related species. Clin. Microbiol. Rev. 6, 324-338.

[15] Drobniewski FA (1994) The safety of *Bacillus* species as insect vector control agents. J. Appl. Bacteriol. 76, 101-109.

[16] Dutky SR (1963) The milky diseases. pp. 75-115 *In:* Steinhaus EA, Ed) *Insect Pathology: An Advanced Treatise. Vol. 2.* Academic Press, New York.

[17] Eidt DC (1985) Toxicity of *Bacillus thuringiensis* var. *kurstaki* to aquatic insects. Can. Entomol. 117, 829-837.

[18] Elliott LJ, Sokolow R, Heumann M & Elefant SL (1988) An exposure characterization of a large scale application of a biological insecticide, *Bacillus thuringiensis*. Appl. Ind. Hyg. 3, 119-122.
[19] Fisher R & Rosner L (1959) Toxicology of the microbial insecticide, Thuricide. Agr. Food Chem. 7, 686-688.
[20] Gill SS, Cowles EA & Pietrantonio PV (1992) The mode of action of *Bacillus thuringiensis* endotoxins. Annu. Rev. Entomol. 37, 615-636.
[21] Giroux S, Coderre D, Vincent C & Cote JC (1994) Effects of *Bacillus thuringiensis* var. *san diego* on predation effectiveness, development and mortality of *Coleomegilla maculata lengi* (Col.:Coccinellidae) larvae. Entomophaga. 39, 61-69.
[22] Green M, Heumann M, Sokolow R, et al. (1990) Public health implications of the microbial pesticide *Bacillus thuringiensis*: an epidemiological study, Oregon, 1985-1986. Am. J. Publ. Health 80, 848-852.
[23] Hadley WM, Burchiel SW, McDowell TD, et al. (1987) Five-month oral (diet) toxicity/infectivity study of *Bacillus thuringiensis* insecticides in sheep. Fund. Appl. Toxicol. 8, 236-242.
[24] Heimpel AM (1971) Safety of insect pathogens for man and vertebrates. pp. 469-489. *In* Microbial Control of insects and mites. (Burges, HD & Hussey NW eds.). Academic Press, New York.
[25] Herms CP, McCullough DG, Bauer LS, et al. (1997) Susceptibility of the endangered Karner blue butterfly (Lepidoptera: Lycaenidae) to *Bacillus thuringiensis* var. *kurstaki* used for gypsy moth suppression on Michigan. Great Lakes Entomol. 30, 125-141.
[26] Hershey AE, Lima AR, Niemi GL & Regal RR (1998) Effects of *Bacillus thuringiensis israelensis* (BTI) and methoprene on nontarget macroinvertebrates in Minnesota wetlands. Ecol. Appl. 8, 41-60.
[27] Hershey AE, Shannon L, Axler R, Ernst C, Michelson P (1995) Effects of methoprene and *Bti* (*Bacillus thuringiensis* var. *israelensis*) on non-target insects. Hydrobiologia 308, 219-227.
[28] Hernandez E, Ramisse F, Ducoureau JP, Cruel T & Cavallo JD (1998) *Bacillus thuringiensis* subsp. *konkukian* (Serotype H34) superinfection: case report and experimental evidence of pathogenicity in immunosuppressed mice. J. Clin. Microbiol. 36, 2138-2139.
[29] Hilbeck A, Baumgartner M, Fried PM & Bigler F (1998) Effects of transgenic *Bacillus thuringiensis* corn-fed prey on mortality and development time of immature *Chrysoperla carnea* (Neuroptera: Chrysopidae). Environ. Entomol. 27, 480-487.
[30] Holmes SB (1998) Reproduction and nest behavior of Tennessee warblers *Vermivora peregrina* in forests treated with Lepidoptera-specific insecticides. J. Appl. Ecol. 35, 185-194.
[31] Hougard J-M, Yaméogo L, Skékétéli A, Boatin B & Dadzie KY (1997) Twenty-two years of blackfly control in the Onchocerciasis Control Porgramme in West Africa. Parasitol. Today 13, 425-431.
[32] Hough-Goldstein J & Keil CB (1991) Prospects for integrated control of the Colorado potato beetle (Coleoptera: Chrysomelidae) using *Perillus bioculatus* (Hemiptera: Pentatomidae) and various pesticides. J. Econ. Entomol. 84, 1645-1651.
[33] Ignoffo CM (1973) Effects of entomopathogens on vertebrates. Ann. NY Academ. Sci. 217, 141-164.
[34] Ignoffo CM (1992) Environmental factors affecting persistence of entomopathogens. Florida Entomol. 75, 516-525.

[35] Innes DG & Bendell JF (1989) The effects of small-mammal populations of aerial applications of *Bacillus thuringiensis*, fenitrothion, and Matacil® used against jack pine budworm in Ontario. Can. J. Zool 67, 1318-1323.

[36] Jackson JK, Sweeney BW, Bott TL, Newbold JD & Kaplan LA (1994) Transport of *Bacillus thuringiensis* var. *israelensis* and its effect on drift and benthic densities of nontarget macroinvertebrates in the Susquehanna River, Northern Pennsylvania. Can. J. Fish. Aquar. Sci. 51, 295-314.

[37] Jackson SG, Goodbrand RB, Ahmed R & Kasatiya S (1995) *Bacillus cereus* and *Bacillus thuringiensis* isolated in a gastroenteritis outbreak investigation. Lett. Appl. Microbiol. 21, 103-105.

[38] James RR, Miller JC & Lighthart B (1993) *Bacillus thuringiensis* var. *kurstaki* afects a beneficial insect, the cinnabar moth (Lepidoptera: Arctiidae). J. Econ. Entomol. 86, 334-339.

[39] Johnson KS, Scriber JM, Nitao JK & Smitley DR (1995) Toxicity of *Bacillus thuringiensis* var. *kurstaki* to three nontarget Lepidoptera in field studies. Environ. Entomol. 24, 288-297.

[40] Karch S, Monteny N, Jullien JL, Sinegre G & Coz J (1990) Control of *Culex pipiens* by *Bacillus sphaericus* and role of nontarget arthropods in its recycling. J. Amer. Mosq. Control Assoc. 6, 47-54.

[41] Koppenhöfer AM & Kaya HK (1997) Additive and synergistic interaction between entomopathogenic nematodes and *Bacillus thuringiensis* for scarab grub control. Biol. Contr. 8, 131-137.

[42] Kreutzweiser DP, Holmes SB, Capell SS & Eichenberg DC (1992) Lethal and sublethal effects of *Bacillus thuringiensis* var. *kurstaki* on aquatic insects in laboratory bioassays and outdoor stream channels. Bull. Environ. Contam. Toxicol. 49, 252-258.

[43] Krieg A & Langenbruch GA (1981) Susceptibility of arthropod species to *Bacillus thuringiensis*. pp. 837-896 *In:* Burges HD, Ed., *Microbial control of pests and plant diseases 1970-1980.* Academic Press, London.

[44] Lacey LA & Dame DA (1982) The effect of *Bacillus thuringiensis* var. *israelensis* on *Toxorhynchites rutilus rutilus* in the presence and absence of prey. J. Med. Entomol 19, 593-596.

[45] Lacey LA, Escaffre H, Philippon B, Sékétéli A & Guillet P (1982) Large river treatment with *Bacillus thuringiensis* (H-14) for the control of *Simulium damnosum* in the Onchocerciasis Control Programme. Z. Tropenmed. Parasitol. 33, 97-101.

[46] Lacey LA, Horton DR, Chauvin RL & Stocker JM (1999) Comparative efficacy of *Beauveria bassiana*, *Bacillus thuringiensis*, and aldicarb for control of Colorado potato beetle in an irrigated desert agroecosystem and their effects on biodiversity. Ent. Exp. Appl. *submitted*.

[47] Lacey LA & Mulla MS (1990) Safety of *Bacillus thuringiensis* (H-14) and *Bacillus sphaericus* to non-target organisms in the aquatic environment. pp. 169-188 *In*: Laird M, Lacey LA & Davidson EW, Eds., *Safety of Microbial Insecticides*. CRC Press, Boca Raton.

[48] Lacey LA & Undeen AH (1986) Microbial control of black flies and mosquitoes. Ann. Rev. Entomol. 31, 265-296.

[49] Laird M, Lacey LA & Davidson EW (Eds.) 1990. *Safety of Microbial Insecticides*. CRC Press, Boca Raton. 259 pp.

[50] Lamanna C & Jones L (1963) Lethality for mice of vegetative and spore forms of *Bacillus cereus* and *Bacillus cereus*-like insect pathogens injected intraperitoneally and subcutaneously. J. Bact. 85, 532-535.

[51] Langenbruch GA (1992) Experiences with *Bacillus thuringiensis* subsp. *tenebrionis* in controlling the Colorado potato beetle. Mitt. der Deutschen Gesellschaft. 8, 193-195.
[52] Larget I & Charles JF (1982) Etude de l'activité larvicide de *Bacillus thuringiensis* variété *israelensis* sur les larves de Toxorhynchitinae. Bull. Soc. Path. Exp. 75, 121-130.
[53] MacIntosh SC, Stone TB, Sims SR, et al. (1990) Specificity and efficacy of purified *Bacillus thuringiensis* proteins against agronomically important insects. J. Invertebr. Pathol. 56, 258-266.
[54] Martin PAW & Travers RS (1989) Worldwide abundance and distribution of *Bacillus thuringiensis* isolates. Appl. Environ. Microbiol. 55, 2437-2442.
[55] Meadows MP (1993) *Bacillus thuringiensis* in the environment: ecology and risk assessment. pp 193-220 *In:* Entwistle PF, Cory JS, Bailey MJ & Higgs H, Eds., *Bacillus thuringiensis, An Envrionmenntal Biopesticide: Theory and Practice*. John Wiley & Sons, Chichester.
[56] Melin BE & Cozzi EM (1990) Safety to nontarget invertebrates of lepidopteran strains of *Bacillus thuringiensis* and their β−exotoxins. pp. 149-167 *In*: Laird M, Lacey LA & Davidson EWD, Eds., *Safety of Microbial Insecticides*. CRC Press, Boca Raton.
[57] Menon AS & DeMestral J (1985) Survival of *Bacillus thuringiensis* var. *kurstaki* in waters. Water, Air, Soil Poll. 25, 265-274.
[58] Merritt RW, Walker ED, Wilzbach MA, Cummins KW & Morgan WT (1989) A broad evaluation of B.t.i. for black fly (Diptera: Simuliidae) control in a Michigan river: efficacy, carry and nontarget effects on invertebrates and fish. J. Am. Mosq. Cont. Assoc. 5, 397-415.
[59] Merritt RW, Wipfli MS & Wotton RS (1991) Changes in feeding habits of selected nontarget aquatic insects in response to live and *Bacillus thuringiensis* var. *israelensis* de Barjac-killed black fly larvae (Diptera: Simuliidae). Can. Entomol. 123, 179-185.
[60] Miller JC (1990) Field assessment of the effects of a microbial pest control agent on nontarget Lepidoptera. Am. Entomol. 36, 135-139.
[61] Molloy DP (1992) Impact of the black fly (Diptera: Simuliidae) control agent *Bacillus thuringiensis* var. *israelensis* on chironomids (Diptera: Chironomidae) and other non target insects: results of ten field trials. J. Am. Mosq. Control Assoc. 8, 24-31.
[62] Mulla MS, Axelrod H, Darwazeh HA & Matanmi BA (1988) Efficacy and longevity of *Bacillus sphaericus* 2362 formulations for control of mosquito larvae in dairy wastewater lagoons J. Am. Mosq. Control Assoc. 4, 448-452
[63] Mulla MS, Darwazeh HA, Davidson EW, Dulmage HT & Singer S (1984) Larvicidal activity and field efficacy of *Bacillus sphaericus* strains against mosquito larvae and their safety to nontarget organisms. Mosq. News 44, 336-342.
[64] Nagy LR & Smith KG (1997) Effects of insecticide-induced reduction in lepidopteran larvae on reproductive success of hooded warblers. The Auk 114, 619-627.
[65] Nealis V & van Frankenhuyzen K (1990) Interactions between *Bacillus thuringiensis* Berliner and *Apanteles fumiferanae* Vier. (Hymenoptera: Braconidae), a parasitoid of the spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). Can. Entomol. 122, 585-594.
[66] Neri-Barbosa JF, Quiros-Martinez H, Rodriguez-Tovar ML, Tejada LO & Badii MH (1997) Use of Bactimos briquets (B. t. i. formulation) combined with the backswimmer *Notonecta irrorata* (Hemiptera: Notonectidae) for control of mosquito larvae. J. Amer. Mosq. Control Assoc. 13, 87-89.
[67] Nicolas L, Dossou-Yovo J & Hougard J-M (1987) Persistence and recycling of *Bacillus sphaericus* 2362 spores in *Culex quinquefasciatus* breeding sites in West Africa. Appl. Microbiol. Biotech. 25, 341-345.

[68] Noble M A, Riben PD & Cook GJ (1992) Microbiological and epidemiological surveillance programme to monitor the health effects of Foray 48B BTK spray. Ministry of Forests, Province of British Columbia.
[69] Obenchain FD & Ellis B-J (1990) Safety considerations in the use of *Bacillus popilliae*, the milky disease pathogen of Scarabaeidae. pp. 189-201 *In*: Laird M, Lacey LA & Davidson EW, Eds., *Safety of Microbial Insecticides*. CRC Press, Boca Raton.
[70] Peacock JW, Schweitzer DF, Carter JL & Dubois NR (1998) Laboratory assessment of the effects of *Bacillus thuringiensis* on native Lepidoptera. Environ. Entomol. 27, 450-457.
[71] Petras SF & Casida LE, Jr (1985) Survival of *Bacillus thuringiensis* spores in soil. Appl. Environ. Microbiol. 50, 1496-1501.
[72] Rodenhouse NL & Holmes RT (1992) Results of experimental and natural food reductions for breeding black-throated blue warblers. Ecology 73, 357-372.
[73] Sample BE, Butler L, Zivkovich C, Whitmore RC & Reardon R (1996) Effects of *Bacillus thuringiensis* Berliner var. *kurstaki* and defoliation by the gypsy moth [*Lymantria dispar* L. (Lepidoptera: Lymantriidae)] on native arthropods in West Virginia. Can. Entomol. 128, 573-592.
[74] Samples JR & Buettner H (1983) Ocular infection caused by a biological insecticide. J. Inf. Dis. 148, 613-614.
[75] Schnepf E, Crickmore N, Van Rie J, et al. (1998) *Bacillus thuringiensis* and its pesticidal proteins. Microbiol. Mol. Biol. Rev. 62, 775-806.
[76] Sebesta K, Fargas J, Horska K & Vankova J (1981) Thuringiensin, the Beta-exotoxin of *Bacillus thuringiensis*. pp. 249-281 *In*: Burges HD, Ed., *Microbial control of pests and plant diseases 1970-1980.* Academic Press, London.
[77] Shadduck JA (1983) Some considerations on the safety evaluation of nonviral microbial pesticides. Bull. WHO 61, 117-128.
[78] Shinagawa K (1990) Purification and characterization of *Bacillus cereus* enterotoxin and its application to diagnosis. pp. 181-193. *In* Toxin in foods and feeds (Pohland AE & Dowell VR, Jr., eds.). Plenum Press, New York.
[79] Siegel JP & Shadduck JA (1990) Mammalian safety of *Bacillus thuringiensis israelensis*. pp. 202-217. *In:* de Barjac H & Sutherland DJ, Eds., *Bacterial control of mosquitoes & black flies: biochemistry, genetics & applications of Bacillus thuringiensis israelensis and Bacillus sphaericus.* Rutgers University Press, New Brunswick.
[80] Siegel JP & Shadduck JA (1990) Mammalian safety of *Bacillus sphaericus*. pp. 321-331. *In:* de Barjac H & Sutherland DJ, Eds., *Bacterial control of mosquitoes & black flies: biochemistry, genetics & applications of Bacillus thuringiensis israelensis and Bacillus sphaericus*. Rutgers University Press, New Brunswick.
[81] Siegel JP & Shadduck JA (1990) Clearance of *Bacillus sphaericus* and *Bacillus thuringiensis* subsp. *israelensis* from mammals. J. Econ. Entomol. 83, 347-355.
[82] Siegel JP, Smith AR & Novak RJ (1997) Cellular fatty acid analysis of a human isolate alleged to be *Bacillus sphaericus* and *Bacillus sphaericus* isolated from a mosquito larvicide. J. Appl. Environ. Microbiol. 63, 1006-1010.
[83] Sims SR (1995) *Bacillus thuringiensis* var. *kurstaki* [CryIA (c)] protein expressed in transgenic cotton: effects of beneficial and other non-target insects. Southwest. Entomol. 20, 493-500.
[84] Sims SR (1997) Host activity spectrum of the cryIIa *Bacillus thuringiensis* subsp. *kurstaki* protein: effects on Lepidoptera, Diptera, and non-target arthropods. Southwest. Entomol. 22, 395-404.

[85] Smirnoff WA & MacLeod CF (1961) Study of the survival of *Bacillus thuringiensis* var. *thuringiensis* Berliner in the digestive tracts and in feces of a small mammal and birds. J. Insect Pathol. 3, 266-270.
[86] Smith RA & Couche GA (1991) The phylloplane as a source of *Bacillus thuringiensis* variants. Appl. Environ. Microbiol. 57, 311-315.
[87] Snarski VM (1990) Interactions between *Bacillus thuringiensis* subsp. *israelensis* and fathead minnows, *Pimephales promelas* Rafinesque, under laboratory conditions. Appl. Environ. Microbiol. 56, 2618-2622.
[88] Steinhaus EA (1959) On the improbability of *Bacillus thuringiensis* Berliner mutating to forms pathogenic for vertebrates. J. Econ. Entomol. 52, 506-508.
[89] USDA Forest Service (1995) Gypsy moth Management in the United States, Final Environmental Impact Statement. 5 Volumes.
[90] Wagner DL, Peacock JW, Carter JL & Talley SE (1996) Field assessment of *Bacillus thuringiensis* on nontarget Lepidoptera. Environ. Entomol. 25, 1444-1454.
[91] Walton WE & Mulla MS (1991) Integrated control of *Culex tarsalis* larvae using *Bacillus sphaericus* and *Gambusia affinis*: Effects on mosquitoes and nontarget organisms in field mesocosms. Bull. Soc. Vector Ecol. 16, 203-221.
[92] Warren RE, Rubenstein D, Ellar DJ, Kramer JM & Gilbert RJ (1984) *Bacillus thuringiensis* var. *israelensis*: protoxin activation and safety. Lancet 1 (8378), 678-679.
[93] Webb RE, Shapiro M, Podgwaite JD, et al. (1989) Effect of aerial spraying with Dimilin, Dipel, or Gypchek on two natural enemies of the gypsy moth (Lepidoptera: Lymantriidae). J. Econ. Entomol. 82, 1695-1701.
[94] Weseloh RM, Andreadis TG, Moore REB, et al. (1983) Field confirmation of a mechanism causing synergism between *Bacillus thuringiensis* and the gypsy moth parasitoid, *Apanteles melanoscelus*. J. Invertebr. Pathol. 41, 99-103.
[95] Whaley WH, Anhold J & Schaalje GB (1998) Canyon drift and dispersion of *Bacillus thuringiensis* and its effects on select nontarget lepidopterans in Utah. Environ. Entomol. 27, 539-548.
[96] Wipfli MS & Merritt RW (1994) Disturbance to a stream food web by a bacterial larvicide specific to black flies: feeding responses of predatory macroinvertebrates. Freshwater Biol. 32, 91-103.
[97] Wipfli MS & Merritt RW (1994) Effects of *Bacillus thuringiensis* var. *israelensis* on nontarget benthic insects through direct and indirect exposure. J. N. Am. Benthol. Soc. 13, 190-205.
[98] Wipfli MS, Merritt RW & Taylor WW (1994) Low toxicity of the black fly larvicide *Bacillus thuringiensis* var. *israelensis* to early stages of brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), and steelhead trout (*Oncorhynchus mykiss*) following direct and indirect exposure. Can. J. Fish. Aquat. Sci. 51, 1451-1458.
[99] Woods SA & Elkinkton JS (1988) Effects of *Bacillus thuringiensis* treatments of the occurrence of nuclear polyhedrosis virus in gypsy moth (Lepidoptera: Lymnatriidae) populations. J. Econ. Entomol. 81, 1706-1714.
[100] Yousten AA (1973) Affect of the *Bacillus thuringiensis* δ–endotoxin on an insect predator which has consumed intoxicated cabbage looper larvae. J. Invertebr. Pathol. 21, 312-314.
[101] Yousten AA, Benfield EF, Campbell RP, Foss SS & Genthner FJ (1991) Fate of *Bacillus sphaericus* 2362 spores following ingestion by nontarget invertebrates. J. Invertebr. Pathol. 58, 427-435.